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A SOIL-BORNE VIRUS DISEASE OF CHERRIES IN THE NETHERLANDS

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A soil-borne virus disease of cherries in the Netherlands

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Introduction

In 1951 MULDER (4) described a virus disease occurring in cherries in the Netherlands and known there as "Eckelrader ziekte" of cherry. Later the identity of the disease with the "Pfeffinger-krankheit", occuring in Switzerland, was generally assumed. In 1959 PFAELTZER (5) proposed the name of "rozetziekte" for the Netherlands as it was thought to be undesirable to name a disease after the locality where it was found for the first time.

Infected cherry trees display both primary and secondary symptoms. The primary symptoms are the so-called oil flecks and malformation of the leaves. The secondary symptoms consist of small, narrow, sharply dentated leaves and of enations at the lower side of some of the leaves. Eventually rosettes of leaves are visible at the end of those twigs that fail to elongate normally.

The "rozetziekte" causes considerable damage in the orchards of the province of Limburg. As the disease was and still is spreading in that area the danger existed that the virus might spread to northern parts of the country. For these reason it was considered to be important to know how the virus spreads in the field. EVENHUIS (2, 3) carried out a number of experiments to establish whether insects, particularly leafhoppers, were able to transmit the causal virus. These experiments, however, all gave negative results. BLUMER (1) and others suggested that the virus might be transmitted by root contact. In the following section a number of experiments are described in which we investigated whether soil-transmission is indeed possible.

Experiments on soil-transmission of the virus

I. In Limburg

Three experiments were carried out with seedlings of *Prunus avium* in a diseased orchard in Limburg. In the following paragraphs these experiments will be described. The results are summarized in table 1.

Experiment A aimed to prove the infection of healthy seedlings when they are planted in an infected orchard. No special measures were taken to prevent the roots from touching the roots of diseased trees, by which possibly root grafting might take place.

In experiment B seedlings were planted in drums filled with healthy and infected soil respectively. Moreover a number of seedlings were planted in the orchard itself. In the drums contact with roots of trees growing outside the drums was of course not possible.

In experiment C ten healthy seedlings, planted in pots were brought from Wageningen to Limburg and placed at random in the orchard to expose them to air-borne vectors. After about one month the plants were changed for fresh ones, whereas the old ones were taken back to Wageningen. Here they were kept in pots, to see whether they had become infected. This was done five times during the summers of 1957 and of 1958. Thus 120 seedlings were used in this experiment.

Table 1 gives the rates of infection in each group of seedlings.

Table 1. Transmission of the "rozetziekte" of cherry under natural conditions

	Experiment	Number of test plants	Number of infected plants
A	in orchard (root contact possible) . . .	9	7
B	in drums with infected soil.	20	5
	in drums with healthy soil	20	0
	in orchard.	20	8
C	in pots (contact with air-borne vectors possible).	120	0

From the symptoms they showed in 1960 it can be concluded that infection of seedlings occurred only when they had grown in infected soil.

II. At Wageningen

In an experiment carried out at Wageningen we used drums filled with infected or uninfected soil, which had been treated in different ways (fig. 1). The treatments were as follows:

Fig. 1. Experiment on soil transmission of the virus of "rozetziekte" of cherry (= Eckelrader disease). Seedlings of *Prunus avium* are planted in infected or uninfected soil which was treated in different ways.

A. Uninfected soil.
B. Uninfected soil to which roots of diseased trees had been added.
C. Infected soil.
D. Infected soil, sterilized by steaming.
E. Infected soil treated with DD and subsequently aerated by means of spreading and turning at set times during several weeks to evaporate DD.
F. Infected soil treated as E. but without DD.

In each drum three *Prunus avium* seedlings were planted. Each treatment comprised eight drums.

Table 2. Soil transmission of the "rozetziekte" of cherry into seedlings growing in soils treated in different ways

	Conditions of the soil	Treatment	Number of infected plants per drum								
A	uninfected	—	0	0	0	0	0	0	0	0	0
B	uninfected	diseased roots added	0	0	0	0	0	0	0	0	0
C	infected	—	2	2	3	1	3	1	0	3	15
D	infected	sterilized by steaming	0	0	0	0	0	0	2	0	2
E	infected	DD added, then aeration	0	0	0	0	0	0	0	0	0
F	infected	aeration without DD	2	0	0	0	0	0	0	0	2

The results of this experiment, given in table 2, show clearly that untreated, infective soil brought about infection in many of the cherry plants growing in it. The only case in which infection occurred in the sterilized soil must be attributed to incomplete sterilization. No infection took place in the DD-treated soil and in only one drum did infection occur in treatment E, comprising turning and spreading of the soil. Soil taken from an infected orchard keeps its infectivity for at least some months, but it is clear that disturbing the soil (treatment F.) decreased the infectivity considerably.

A plausible conclusion from the results of this experiment is that the virus is transmitted by an organism living in the soil, although adsorption of the virus to the soil particles cannot be excluded.

The nature of the vector is not yet known. It may well be an eelworm as is known to be the case with other soil-borne viruses. Experiments are in progress to investigate this possibility, but these results can only be expected, at the earliest, next year.

In all transmission experiments described above many of the trees not only showed primary symptoms, but also secondary symptoms (fig. 2).

Fig. 2. Symptoms of "rozetziekte" of cherry (= Eckelrader disease) on a seedling of *Prunus avium* growing in untreated infective soil (treatment C). The top leaves show primary symptoms: malformation and oil flecks. The lower leaves show secondary symptoms: They are small, narrow and sharply dentated.

In the orchard we have never found any cherry tree which has developed year after year primary or secondary symptoms only. It seems therefore reasonable to assume that the disease is caused by a single virus and not by a complex of viruses.

In deciding whether or not infection has taken place, we have to rely on the production of symptoms by the plants because there is no method to demonstrate infection until the symptoms become visible. To be sure that a plant did not become infected we have to wait for at least two years.

Hosts of the virus

As was described earlier, it takes a long time before one can establish, by planting healthy seedlings, whether the soil is infected or not. We have therefore tried to find an indicator which might enable us to demonstrate an infection of the soil within a short time. For that purpose several rosaceous plants were planted in an orchard, which was known to be severely infected.

Table 3. Natural infection of different plant species by the "rozetziekte" of cherries

Plant species	Number of test plants	Infected test plants
Morello	10	3
Peach	17	0
Myrobalan	9	0
Prunus mahaleb	4	1
F 12/1 root stock	6	5
Raspberry	5	1
Blackberry	3	2

In table 3 the results of these experiments are given. Morello, *Prunus mahaleb,* F 12/1 root stock, raspberry and blackberry became infected. The symptoms of the infected plants, however, take as long a time to develop as when cherry seedlings are used. They therefore are no better indicators than cherry itself, yet they are of importance because they have to be regarded as possible sources of infection in the soil.

In greenhouse experiments certain herbaceous plants such as tobacco, cucumber, spinach, turnip, beet, plantain and other species were tested for their qualities as indicators. So far no useful results have been obtained.

Identity of the virus

It is very difficult to say whether there is a relationship between the cherry virus described here and the cherry viruses and soil-borne viruses described elsewhere.

Identification of such viruses by comparison of symptoms in cherry trees is impossible in most cases as the exchange of infected plant material is hampered by quarantine measures. Moreover the development of symptoms in trees often takes a long time. For this reason, transmission of the virus involved to herbaceous hosts might be helpful. The virus could be transmitted from cherry to tobacco and cucumber plants and from those to many other herbaceous hosts in all of which the virus became systemic.

In order to prove that the virus involved was indeed the virus which causes the "rozetziekte", inoculations back to cherries have been carried out (6). Cherry seedlings were inoculated by rubbing the young leaves with infectious tobacco sap and carborundum or by injecting the inoculum into the stems. The speed of development of symptoms varied considerably depending on the age of the plants, the mode of inoculation and seasonal influences. The shortest incubation time found was about four weeks; the longest 15 months. The test plants showed typical chlorotic flecks and ring-spots in some of the leaves but until now none of the secondary symptoms could be detected.

The question arises whether in these and in other experiments the cherry ring-spot virus might be involved. This is, however, very unlikely as there has never been any virus transmission to tobacco or cucumber from cherries showing ring-spot symptoms. The chlorotic flecks and rings which developed in the cherry seedlings inoculated with infectious tobacco sap can therefore not be attributed to the cherry ring-spot virus.

The symptoms caused by the virus from "rozet"-diseased cherries in herbaceous hosts vary a great deal. Other viruses such as raspberry ring-spot and raspberry yellow dwarf, which might be related to this virus, cause similar symptoms in one host and differing symptoms in another.

For this reason identifying cherry viruses and determining their relationship with other viruses by means of comparing

symptoms in herbaceous hosts, encounters serious difficulties. Serology will give us a better basis for answering the questions concerning the identity of the virus of the "rozetziekte" of cherries and its relationship to other viruses.

SUMMARY

In 1951 MULDER described the "Eckelrader ziekte", a virus disease of cherries in the Netherlands. In 1959 PFAELTZER proposed to name the disease "rozetziekte" of cherries. In experiments concerning the transmission of the causal virus no air-borne vector could be detected by EVENHUIS.

A number of experiments described here point to the fact that the virus is soil-borne (tables 1 and 2). Experiments are in progress to investigate whether eelworms can transmit the virus.

The virus of the "rozetziekte" infected morello, *Prunus mahaleb*, raspberry, blackberry and rootstock F 12/1 when grown in infected soil under natural conditions (table 3). Alle the plants mentioned developed symptoms only a long time after planting into infective soil and therefore they cannot be considered as good indicator hosts. However, they can be regarded as possible sources of the virus in the soil.

A number of herbaceous plant species could be infected mechanically with the virus, viz. tobacco and cucumber. Inoculation of the virus from tobacco back to cherries caused chlorotic spots and ring-spots in some of the cherry leaves, but secondary symptoms have not yet been observed.

Herbaceous hosts can provide suitable material for the preparation of antisera. The use of antisera may be of great help in establishing relationship amongst a number of the soil-borne viruses known at present.

DISCUSSION

BAUMANN: To avoid confusion in nomenclature clearly differentiation between the virus disease and the causing virus must be made.

Therefore we should not name the well known Eckelrader ziekte as rosette ziekte, because in this disease according to Cadmanns statement not only the cherry rosette virus is involved.

Furthermore the name is not specific for the main symptoms of Pfeffinger and Eckelrader disease and already used for another virus disease of sweet cherry (Blumer 1953).

Kegler: Did you investigate samples of soil from highly infected orchards, and did you find any free living nematodes such as Xiphinema sp.?

Pfaeltzer: We inspected samples from highly infected orchards and found free living nematodes, e.g. a species of Longidorus, but no Xiphinema was found.

Schmid: Neither have we found any Xiphinema in Pfeffinger diseased soils.

Millikan: Did you find enations on leaves of Prunus mechanically inoculated with juice?

When did you expect secondary symptoms to occur?

Pfaeltzer: No secondary symptoms have been detected up to now.

Gualaccini: Did you for the inoculations of the disease into herbaceous host plants use leaf juice only or juice together with any buffer solution?

Pfaeltzer: Only carborundum was added to the plant sap and no buffer was used.

Nyland: Did you make isolation attempts from roots of herbaceous plants which you grew in infected soil?

Pfaeltzer: Yes, but I obtained no transmission.

Kunze: There are some latent viruses in cherries, which are sap-transmissible to cucumber and tobacco, but the longevity of these viruses is shorter than that of the "Pfeffinger virus". It is therefore difficult to transmit such viruses from cherries to herbaceous plants unless buds are used as inoculum.

Raski: A recommendation is made to use the term *migratory plant parasitic nematodes* when referring to the ectoparasitic forms found transmitting virus diseases. Several contributors have used the term "free living" plant parasitic nematodes, which will be confused with the large group of nematode species found in almost all cultivated fields. These have traditionally been called "free living" since they fed on bacteria, fungi and other microorganisms but not higher plants.

Millikan: Have the seedlings which were infected with tobacco sap shown secondary symptoms?

Pfaeltzer: Up till now no secondary symptoms have been found on inoculated cherry seedlings. As the secondary symptoms always appear some time after the primary symptoms it is possible that they will develop next year or even within two years. If they do not appear at all that will confirm the hypothesis of the complex character of the disease.

Posnette: How many trees were inspected on their symptom expression? May be some trees in the field might show only one type of symptoms.

Pfaeltzer: An elaborate investigation on the spread of the disease in orchards of southern Limburg has been carried out by the Plant Protection Service during several years. During this investigation no trees

were found which year after year developed either primary or secondary symptoms only. Of course a tree may show only primary symptoms during the first phase of the disease after infection and during the last phase it may show only secondary symptoms. From this we can draw the conclusion that the factor (or factors) which causes (cause) the two types of symptoms is (are) always present in infected trees.

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